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■ Hints and tips

Reproducibility of UV-research with plants

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Introduction

This is the second installment of what I hope will be a useful, albeit opinionated, regular column in the Bulletin. I aim to focus on important methodological and practical questions that are central to the quality of our research. I will occasionally make diversions into philosophy of science and other subjects relevant to all research activities. Suggestions for subjects to explain or highlight and questions highly welcome.

Lack of reproducibility in scientific research is a broad problem that has woken up the interest of politicians and the general public (Fineberg 2019). For example the US Congress requested the US National Academy of Sciences to produce a report about this problem (Fineberg 2019). Given that eight years have passed since *Beyond the Visible* (Aphalo, Albert, Björn, et al. 2012) was written, it is timely to remind our research community about some problems that keep reappearing both in submitted manuscripts and published articles. I have chosen as the subject of the present column *reproducibility of UV-research with plants*.

In our field of research, the main sources of difficulties seem to be the use of flawed methods, the incomplete description of methods and the misinterpretation of experimental results by ignoring the limitations of the protocols and methods used. Even though rather few papers published in our field are flawed in ways that would require retraction, a very large proportion of papers are unnecessarily weakened in their usefulness and trustworthiness by these problems. In my view, after an initial and significant improvement in the quality of research and reporting during the UV4Growth COST action, in recent years the quality of research and reporting has gradually deteriorated. This is not a phenomenon restricted to low impact journals but affects also very highly ranked journals. Given that flawed papers are being accepted for publication, the problem concerns authors, reviewers and editors.

As this is a column about hints and tips, I will focus mainly on how to avoid

problems that can affect experiments aiming to study responses to UV-B radiation. I will start with the crucial question of what an experiment tests for, continue with other problems that can prevent reproducibility and end with my personal view of why trustworthy scientific research and reproducibility transcend the aims of our own careers.

Controls and treatments

Problem: Use of unsuitable controls and/or treatments and the misinterpretation of the results from badly designed experiments is "bad science", that contributes to inefficient use of resources and contaminates the corpus of shared scientific knowledge.

In any comparative study, controls and treatments are equally important. Control and treatment conditions must be chosen with equal care and described in the same detail. Obviously the quality of what we can infer from their comparison is limited by the weakest of the two. The question of suitable controls in UV research has been already discussed in depth, most frequently in relation to UV-supplementation studies carried out outdoors (Aphalo, Albert, McLeod, et al. 2012; Newsham et al. 1996). The same argumentation concerns laboratory and controlled environments experiments. In all cases, the question is to identify all relevant differences between treatments and controls, and design both controls and treatments in a way that makes the observed effects interpretable.

All light sources have side effects like emission of radiation at wavelengths shorter and longer than UV-B including thermal radiation. Lamps can also shade radiation from other sources and potentially create electromagnetic fields. Broad-band UV-B lamps like the widely used Philips TL12 and Q-Panel UVB313 are not UV-B lamps, they are lamps that emit UV-B radiation as well as UV-C, UV-A, visible and thermal radiation. The UV-B component of the photon emission from these lamps is only about 30% of their total UV plus visible emission. A comparison between the effect of energized "UV-B" lamps vs. no lamps, either outdoors or in the laboratory should never be interpreted as an effect of UV-B radiation. Frequently used pairs of controls vs. treatments are listed in Table 11.1 together with the main differences between them and whether they reliably test for an effect of UV-B radiation or not.

Even if we ignore thermal radiation, possible shading, and other side effects and assess how much of the difference in photon irradiance between control and treatment conditions is in the UV-B band, we obtain values in the range from 33% and 96% (Figure 11.1). The main issue for reproducibility is that these different experimental protocols test for quite different effects: from effects that can be only safely interpreted as a generic effects of a type of lamps to effects that can be rather safely attributed to a specific range of wavelengths. Even within the UV-B band, different wavelengths cannot be expected to be equally effective.

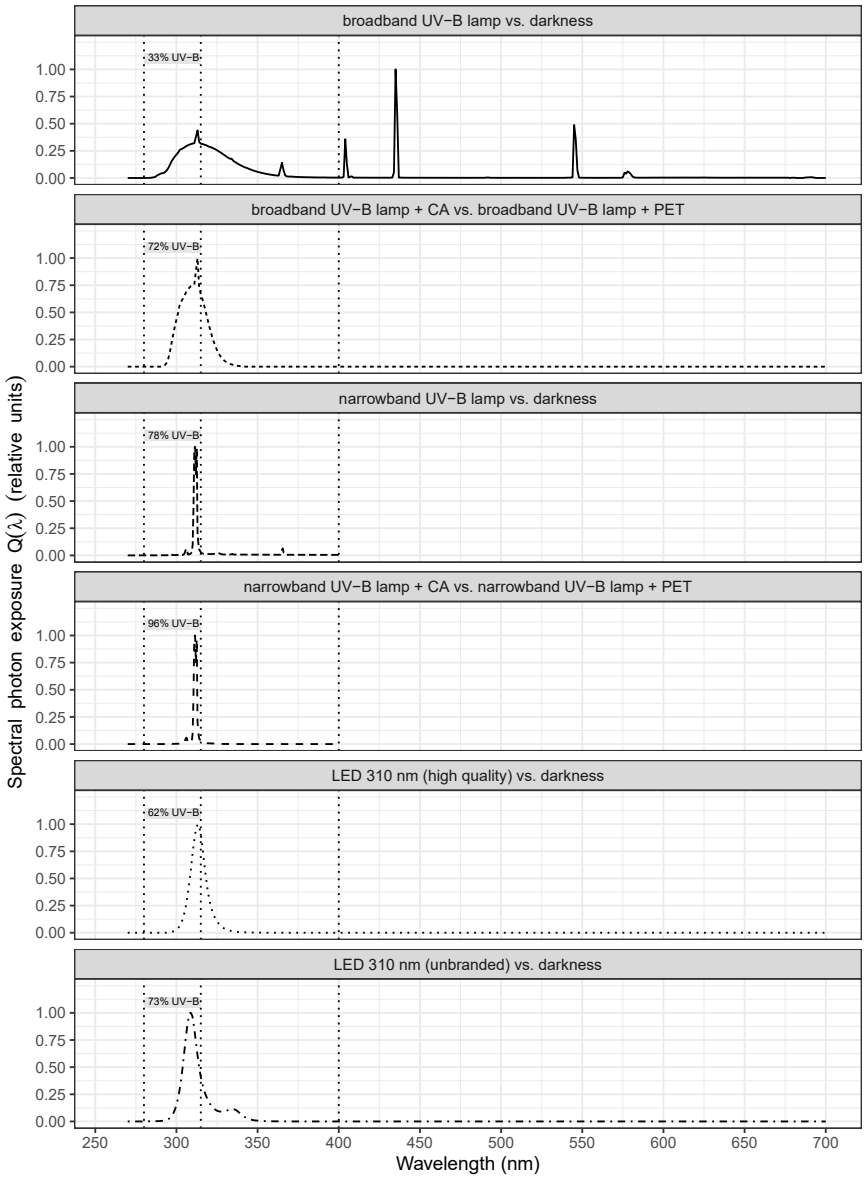

Figure 11.1: Difference spectra for six pairs of UV-B treatment and control conditions in use. The conditions are shown in panel headers as "treatment vs. control". The spectra plotted describe the difference in spectral irradiance between treatment and control in a given pair. Spectra are normalised. The labels show the percentage of UV-B photons compared to the total number photons in the range of wavelengths plotted. The vertical dotted lines show the boundaries of the UV-B and UV-A wavebands. See Appendix for code used.

Table 11.1: Testing for UV-B effects. Pairs of controls and treatments frequently used in experiments reported in the scientific literature to assess responses to ultraviolet-B radiation.

Control	Treatment	Differences	Test for UV-B
darkness	broadband UV-B lamp	UV-C, UV-B, UV-A, VIS, thermal	NO
wb UV-B lamp + PET	wb UV-B lamp + CA	UV-B, (UV-Asw)	YES
darkness	narrowband UV-B lamp	UV-B, (VIS), thermal	(YES)
narrowband UV-B lamp + PET	narrowband UV-B lamp + CA	UV-B	YES
darkness	LED 310 nm (unbranded)	UV-B, (thermal)	(YES)
darkness	LED 310 nm (high quality)	UV-B, UV-Asw, (thermal)	NO

Solution: Always provide information about background illumination and other environmental conditions in as much detail as needed for research to be reproducible and reliably evaluated. Readers should have access to detailed information on both treatment and control conditions including all aspects in which they differ. In the absence of this information it is impossible to assess if the conclusions drawn by the authors of the study are valid. Incomplete description of the test conditions also impedes any attempt to reproduce the experiment. Be careful when drawing conclusions and always inform readers about the limitations of the study and any caveats that may apply.

Background illumination

Problem: A surprisingly large number of papers reporting on experiments carried out in the laboratory fail to mention if the UV-treatments were applied under a background of white light or in darkness. We are also only rarely told under which conditions the controls were kept while the treatments were applied (e.g. same irradiance of visible light, same temperature, etc.). The spectrum and irradiance of the background UV, visible and NIR radiation is almost never reported. The lack of this information makes experiments not reproducible by independent researchers and can easily make results from different studies seem contradictory. This tends to be the result of authors relying on implicit, and frequently unwarranted, assumptions for the interpretation of results, such as "weak background illumination can be ignored".

Does this matter? Yes, because the ratio between different wavelengths affects responses (Krizek 2004; Yan et al. 2020) through signalling interactions downstream of UVR8 and other photoreceptors (Lau et al. 2019; Morales et al. 2015; Moriconi et al. 2018; Rai et al. 2019, 2020; Tissot and Ulm 2020) and because UVR8 can also participate in the perception of UV-A radiation (Rai et al. 2020). Consequently, the interpretation and the range of applicability of the results depends on information about the whole spectrum. Results from

earlier studies that describe methods in enough detail can be re-interpreted in the light of later advances, but those reported with incomplete methods, cannot.

Solution: Always provide information about background illumination and other environmental conditions in as much detail as possible for every single report or article you write. Ensure that you describe in detail any differences in how UV-irradiated and control plants were handled and also what the shared conditions were.

Variation among lamps

Problem: Specifying a lamp type in most cases does not provide enough information. In the long run manufacturers tend to revise the specifications of the lamps they sell without changing the type name or code. There is variation from batch to batch, and for LEDs even between individual LEDs of the same type, so much that many classify them into "bins" or subtypes based on the measured peak wavelength and emission efficiency. Specially the "fantasy names" like "UV-B lamps or UV LEDs" used by some lamp sellers. UV-B, UV-A, and black light broadband lamps all emit visible light and UV-radiation at other wavelengths than those expected from their names. In many cases even codes derived from such names are inconsistently used. The peaks of emission can be at different wavelengths for equivalent lamps from different suppliers (e.g., "black light blue" or BLB lamps have maximum emission at either 385 nm or 368 nm depending on supplier or vintage). In addition the output of both fluorescent lamps and LEDs depends on ambient temperature and on their age.

Be aware that reflections from walls, tables, glass and metal objects, and even clothes can distort the spectrum impinging on plants. Not only reflection is important in the case of UV radiation, many objects fluoresce strongly in the blue or other regions when illuminated with UV radiation, e.g., white paper and clothes, and laundry powders contain fluorescent additives that are added so that paper and clothes look whiter (Björn et al. 2012).

Solution: Whenever possible provide a measured spectrum for the UV source(s) actually used, measured under the same ambient conditions and at the same physical location. Measurements should be done close in time to when the UV sources were used if not at the same time. Do not trust previous measurements or manufacturer specifications.

Petri dishes, microscope cover slides and other barriers

Problem: Rarely the existence or not of a barrier and whether the irradiance or spectra have been measured behind the barrier or in front of it is reported. Even less frequently the exact type and supplier are reported. If light or

UV treatments are applied through the lids of Petri dishes, a cover-slip or microscope slide, a water layer or there is anything else than air in the path of the radiation the spectrum and irradiance could be significantly affected. As we do not see in the UV, what looks transparent, may not be so in UV.

The shape of the barrier or vessel can also make it function as a lens. So irradiation of liquid samples is best done in vessels with a square cross section, i.e., the same reason why spectrophotometry cuvettes are almost never round like normal test tubes.

Solution: Measure (or at least estimate) the spectrum and irradiance and define treatments as received by the target organism behind any barrier that separates it from the light source. Do also remember to take into account that the angle of incidence matters both for glass or plastic barrier and the organism.

Spectral irradiance vs. irradiance

Problem: The definitions of UV-B, UV-A and violet-blue radiation do not coincide with the wavelength regions to which UVR8 and cryptochromes are responsive to (Rai et al. 2020). Conditions described only by UV-B and/or UV-A irradiances or exposures make difficult to interpret the observed responses. Lack of spectral data hinders reproducibility and can prevent the use of the data in meta-analyses,

Solution: Provide spectral data for treatment and control conditions and for growing conditions when reporting results from any photobiological study.

Be distrustful of surprising results

Problem: Over reaction to surprising results. Over-interpretation and too-early dumping of surprising results are embarrassing and wasteful, respectively. Over- and misinterpretation of results are common, specially in those journals that too easily accept newsworthy and controversial reports. In the case of surprising results that are discarded too early we can only guess that this can also easily happen.

Reported values that are incompatible with the description of what and how was measured are worryingly common in publications. One can almost always assess the "sanity" of measured values we obtain. For example molar extinction coefficient values for proteins can roughly and easily be estimated on the basis of the amino acid sequence. This is only an approximation, but if our measured values are nearly two orders of magnitude larger, we should carefully investigate what is going on. If our estimate of water vapour pressure is higher than that expected at 100% relative humidity we should check our instruments. If the UV-B irradiance from our lamps is many times less than what others have reported for the same lamps, filters and distance,

we should check calculations and measuring instruments. These examples are real, and for the last one I know of two cases, due to different problems. Of these four examples two made all the way to publication and two were caught in time.

In the first two cases I have no idea of the cause behind the bad data. In one of last two cases calculation errors were the cause, and in the other case a completely wrong calibration of a new spectrometer, supplied by the manufacturer caused the problem. This may sound disappointing, but in my experience, most unusual and surprising results from routine measurements using usual methods and applying similar treatments as others have earlier used are usually caused by methodological problems and mistakes.

On the other hand, surprising results can be real, and tell an unexpected story, even if caused by mistakes. Deeper problems are caused by jumping to conclusions too easily.

Solution: Be distrustful of any results, specially those that seem too good or too bad to be true. Cool down your enthusiasm or despair, imagine yourself for a while as an external reviewer, picky and suspicious of everything. Ahh…do remember to switch back to your positive and enthusiastic self once you have checked your data and before you deal with the problems you may have found!

Reproducibility and correction of past errors

Problem: The self-correction mechanisms of science are made sluggish by the persistence of misconceptions and the continued use of methods known to be bad (due to tradition?), e.g., the recent growth in popularity of Arnon's equation for quantification of chlorophyll concentration by spectrophotometry, even though it has been known for well over three decades that it yields wrong estimates of the concentration (Porra and Scheer 2018).

Scientific knowledge advances by the revision and correction of previous theories and hypotheses (see Godfrey-Smith 2003, for an introduction to the phylosophy of science). This concerns science as a whole, but also each one of us. We develop as researchers and advance in our career by the same process. There is no shame or problem in changing our opinion and we should be open about these changes. If you are a young researcher, *do not be afraid of changing your mind during the course of your career.* This is how one grows as a researcher. In the same way that we may want to criticise earlier publications or suggest changes or replacements for views from other authors we should be ready to criticise and revise the ideas we have proposed in our earlier publications.

A research report usually contributes data and ideas. These are linked, and this link builds upon earlier ideas and data. Depending on the case, the data or the ideas will be relevant for a longer time, while the link between them will frequently become outdated first. Data for which methods are incom-

plete has little value in itself, as it cannot be reinterpreted in the light of new ideas. Conclusions and knowledge that is built upon evidence whose strength or reliability cannot be independently assessed contribute little to scientific progress. If they guide subsequent research unnecessarily into conceptual dead-ends they disturb the normal progress of scientific research frequently causing expensive distraction of resources.

So, we should strive as a community to retroactively correct if possible or alternatively highlight the flaws and inaccuracies in our own and in other authors' publications, both old and recent. This is simply how science is supposed to self-correct errors and we should not be afraid of doing so.

Solution: Both in terms of career progress and contribution to society avoid thinking only in the short term or with a narrow view. More broadly, the evaluation and rewards systems used for scientific research need to be reformulated so that the premium for doing reproducible and useful scientific research vs. flashy and unwarranted controversial or hastily done but over-interpreted studies is very clearly in favour of the first.

Coda: Research is expensive, but justified based on the benefits it can provide to our society. Bad science derails decision making and biases resource allocation. Even if "bad science", intentional and accidental, has a much more direct and dramatic impact in medicine and health care (Goldacre 2010) than in our research field, the same principles apply and are applicable to efforts to improve plant production and food security (Sadras et al. 2020). There is constant tension in the allocation of funding to research, as most research ultimately competes for taxpayers' money that could be used to improve voters' wellbeing in other more direct ways. We ensure that our work provides the maximum benefit to society if the fruits of our work can be trusted and the quality and relevance of the data we generate can be properly and independently assessed. Transfer of knowledge to stakeholders is a crucial step, but first we need to generate knowledge that stakeholders can trust and use with benefit.

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Appendix

Source code of the R script used to create Figure 11.1 which uses data and functions published as part of the *R for photobiology* suite (Aphalo 2015).

```
library(photobiology)
library(photobiologyLamps)
library(photobiologyLEDs)
library(photobiologyFilters)
library(photobiologyWavebands)
library(ggspectra)
library(wrapr)

photon_as_default()

list("broadband UV-B lamp vs.\ darkness" =
       lamps.mspct$qpanel.uvb313,
     "broadband UV-B lamp + CA vs.\ broadband UV-B lamp + PET" =
       lamps.mspct$qpanel.uvb313 * filters.mspct$Courtaulds_CA_115um_age020 -
     lamps.mspct$qpanel.uvb313 * filters.mspct$McDermit_PET_Autostat_CT5_125um,
     "narrowband UV-B lamp vs.\ darkness" =
       lamps.mspct$philips.tl01,
```

```
    "narrowband UV-B lamp + CA vs.\ narrowband UV-B lamp + PET" =
      lamps.mspct$philips.tl01 * filters.mspct$Courtaulds_CA_115um_age020 -
     lamps.mspct$philips.tl01 * filters.mspct$McDermit_PET_Autostat_CT5_125um,
    "LED 310 nm (high quality) vs.\ darkness" =
      leds.mspct$UVMAX305,
    "LED 310 nm (unbranded) vs.\ darkness" =
      leds.mspct$TY_UV310nm
) %.>%
  source_mspct(.) %.>%
  clean(.) %.>%
  normalise(.) %.>%
  autoplot(., range = c(270, 700), annotations = list(c("-", "labels"),
                                                      c("+", "reserve.space"))) +
  stat_wb_box(w.band = UVB(),
              ymin = 1.05, ymax = 1.15, fill = "grey90") +
  stat_wb_contribution(w.band = UVB(), label.mult = 1e2,
                       ypos.fixed = 1.11,
                       label.fmt = "%3.0f%% UV-B", size = 2, color = "black") +
  geom_vline(xintercept = c(280, 315, 400), linetype = "dotted") +
  facet_wrap(~spct.idx, ncol = 1) +
  theme(legend.position = "none")
unset_radiation_unit_default()
```

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